IRON-LABELED NEURAL STEM CELL HOMING AFTER NEONATAL HYPOXIA-ISCHEMIA

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Introduction

Neural stem cells (NSCs) have attracted increasing attention as a potential treatment for neonatal hypoxic ischemic injury (HII). Recently, several studies have demonstrated a reparative effect on brain injury in adult rats after hypoxia ischemia. Although multiple mechanisms, aside from neural replacement, have been proposed to explain this reparative effect, it is realized that non-invasive monitoring of NSC migration, homing and integration to the site of injury would provide an understanding of the injury repair mechanisms.

Methods

Neural Stem Cells: C17.2 NSCs were generously provided by Snyder, have been engineered to express GFP and their ability to engraft and integrate has been previously documented[1]. NSCs are prepared (4-10 x 10^4 cells/µl in PBS with 0.05% trypan blue) for grafting as previously described[1]. Iron labeling was performed 24hrs prior to implantation using Feridex (30ug/4ml) which yielded 80-85% labeling efficiency. Neonatal Hypoxia Ischemia and NSC Implantation: Neonatal hypoxia ischemia was induced in 10 day old rat pups using unilateral carotid ligation/8% hypoxia (n=3, Rice-Vannucci model, RVM) as previously described. Three days after RVM induction, we implanted iron-labeled NSCs (500K in 5uL) into the contralateral ventricle or into the contralateral parenchyma; controls (n=3) without RVM were also injected. After implantation animals were allowed to recover and imaging was performed 24hrs later. Neuroimaging and Analysis: All 10-day old RVM and control rat pups where imaged using diffusion weighted imaging (DWI) and T2WI to monitor ischemia and NSC progression in an 11.7 T scanner (Bruker, Bruker Biospin). NSC migration was tracked for up to 41 weeks using T2WI. Analysis consisted for 3D volumetric reconstructions of 1) whole brain volume, 2) ischemic tissue volume, and 3) NSC volume and location using Amira (Mercury Computer Systems) software.

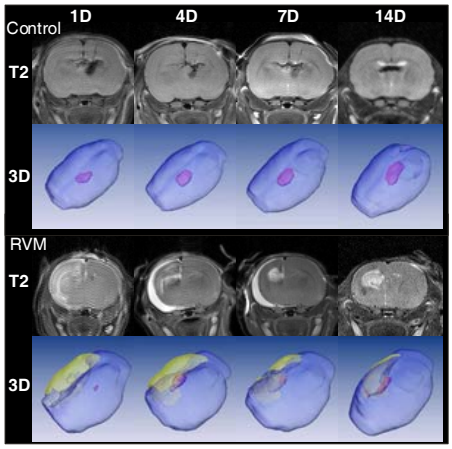

Fig 1: Neuroimaging and 3D reconstruction after NSC injection into the contralateral ventricle at 1,4,7 & 14d. Using both T2 and DWI datasets temporal evaluation of SC migration was assessed in control (no HI) and ischemic animals (RVM). NSCs (**purple**) did not migrate out of the ventricles in control animals. However, in animals after moderate HI (**yellow**) the focally-injected NSCs slowly migrate to the contralateral injured hemisphere. On T2 images these changes are noted as hypointensities

Results

Neuroimaging of the ischemic tissue was readily observed using DWI while iron labeled NSCs were visualized with T2WI (Fig 1). In control animals NSCs remained within the ventricle while in the RVM pups there was a rapid migration of NSCs towards the area of ischemic injury within 4 days and continued over the 41 week period of study. Quantification of the speed of migration revealed that these NSC can travel to the site of HII at up to 600um/day during the first week, while there is no movement of NSCs in control animals (Fig.1 & 2). The MRI-derived NSC volumes at 4d after implantation were 2.2±0.15% and 2.3±0.50% of the total brain volume for controls and HII, respectively. By 21 weeks the NSC volumes had declined to 0.8±0.03% in controls and to 1.0±0.82% in HII. The temporal change in the apparent initial volume of NSCs changed over time, suggests that the NSCs spread out towards different regions of ischemic injury, possibly in response to different homing signals released by injured tissue.

Conclusion

Our findings demonstrate that iron-labeled NSCs can non-invasively be tracked for extended periods of time in a rat pup RVM model and that such methods can be used to develop the optimal method for NSC implantation to maximize recovery after neonatal HII.

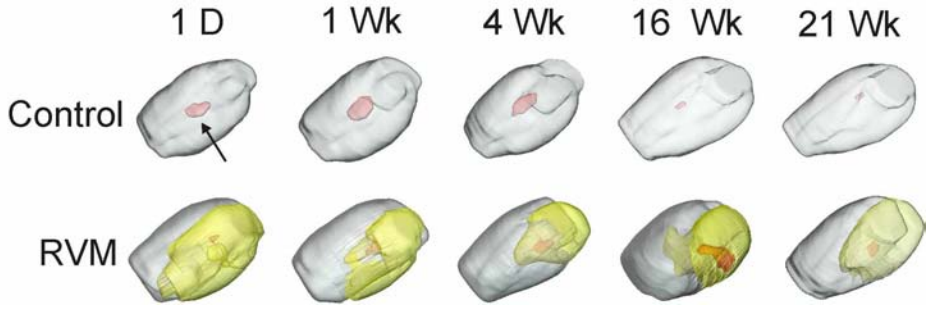

Fig 2: Serial neuroimaging highlights regional and temporal changes in control and RVM animals. Serial T2- weighted and diffusion weighted images were reconstructed for volumetric and regional analysis. In the control animal, iron labeled hNSC (red, arrow) injected into the ventricle remained visible for 21 weeks and did not migrate to other regions of the brain. In contrast, after Ischemic injury (yellow) can be clearly seen at 1 day that encompasses 40% of the hemisphere. Contralateral injection of hNSCs resulted in rapid (within 1wk) migration to the injured tissues. The iron labeled hNSC (red) were clearly visible within the lesion. MRI coronal sections showed what appeared to be integration into the injured tissues (compare 1d with 16wk) but this awaits further histological analysis.

1. Imitola J, Raddassi K, Park KI et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1{alpha}/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A. 2004; 101:18117-18122